LIMITATIONS OF BIOIMPEDANCE-BASED AUTHENTICATION OF PREVIOUSLY FROZEN BEEF– EFFECTS OF SHELF LIFE AND FREEZING TEMPERATURES

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I. INTRODUCTION

European food regulations stipulate that previously frozen, unprocessed meat must be labelled as 'defrosted' and with the date of initial freezing. However, no standard test for detection of freeze damage [1] and for the authentication of refrigerated ('fresh') versus defrosted meat is established yet by food authorities. While several methods, including bioimpedance spectroscopy [2], were proposed for 'fresh meat' authentication, data is scarce regarding the detection capacity and the limitations of each method. Specifically, previous studies often use relatively fresh, post-slaughter meat as the refrigerated-only control. Yet, methods for refrigerated versus defrosted authentication should ideally separate such treatment groups throughout the entire product shelf life, despite inevitable quality deterioration also with longer refrigerated storage. In addition, it is often not clear how different freezing temperatures may compromise a method's detection capacity for fraudulent labelling. In particular, this is the case, for high sub-zero temperatures above -18°C, i.e., for freezing temperatures that are not typically employed by companies following common industry standards for 'quick frozen' labelling. For pork, we have previously established a high detection capacity of bioimpedance-based testing for defrosted meat throughout the entire refrigerated shelf life of about 12 days [2]. Extending on this, we here ask, how the detection capacity of bioimpedance-based detection of beef may be affected by extended refrigerated shelf life and by exposing meat to higher sub-zero freezing treatments.

II. MATERIALS AND METHODS

Meat samples (topside muscle, *M. semimembranosus*, 5 x 5 x 4cm^3) were obtained from a total of 24 animals (*Bos taurus*) and about 24-hour post-mortem. After initial testing for bioimpedance response (day 0) and common physicochemical parameters (pH, CIELAB, temperature), 5 samples from each individual were subjected to 5 different storage treatments: 2°C ('refrigerated only'), -4°C, -14°C, -24°C, -80°C. After 21days, including 2 days thawing period for frozen groups, all samples were measured again. For bioelectrical impedance testing we used a Zurich instruments MFIA impedance analyzer (Zurich Instruments AG, Switzerland). A tetrapolar electrode measurement system was applied with stainless-steel electrodes (2 mm diameter, 12 mm length, 18 mm distance between the middle (voltage) pick-up electrodes). Impedance spectra were recorded for a frequency range from 10Hz to 1MHz with 40 distinct frequency points and with the applied voltage 300 mV rms. For each sample, 4 impedance measurements were taken at defined sample locations. Bioelectrical impedance measurements were made in refrigerated or defrosted beef samples within a temperature range between 2°C and 6°C. Finally, for comparing bioimpedance responses, the P_y parameter [3], a well-established meat quality parameter, was calculated using R_0 and R_∞ (low and high frequency impedance respectively), which were obtained using circular curve fitting to the whole frequency impedance spectra. Statistical analyses were performed with Minitab and included ANOVA, Mann-Whitney U (MWU) and Tukey's testing.

III. RESULTS AND DISCUSSION

First, we tested for overall differences in bioimpedance response, i.e., P_y values between refrigerated-only samples measured early post-mortem and the same samples measured after different freezing treatment (ANOVA, $F_{\text{fresh day 0/-4°C/-14°C/-22°C/-80°C}}=50.53$, $P\leq0.001$). Using pairwise testing for each freeze-treatment revealed a highly significant difference between the fresh and frozen-thawed samples for all treatments (MWU, $P\leq0.001$ for -4°C/-14°C/-22°C and -80°C; table 1). Next, we tested if bioimpedance response also differs between extended refrigerated (2°C) versus frozen storage. An overall comparison revealed a significant effect of storage treatment (ANOVA, $F_{\text{+2°C for 21days/-4°C/-14°C/-22°C/-80°C}}=20.28$, $P\leq0.001$). However, a significant effect was not detected between all individual storage treatments. Specifically, we did not find significant differences between samples stored for 21 days at +2°C versus freeze storage at -4°C (table 1). Yet, differences were detected between either the 2°C or -4°C versus all three lower temperature freeze storage groups (-14°C/-22°C and -80°C; table 1).

Table 1. MWU statistics for all pairwise comparisons of (i) samples measured at day 0 (baseline, before treatment) vs. day 21 (after freeze treatment) and (ii) refrigerated samples kept at 2°C for 21 days versus the four freeze treatments (the respective controls for all comparisons are marked as grey cells).

	day 0 (before treatment)					day 21 (after extended storage treatment)			
treatment	**−4°C**	**−14°C**	**−22°C**	**−80°C**	**2°C**	**−4°C**	**−14°C**	**−22°C**	**−80°C**
day 0 before vs day 21 after treatm.						P≤0.001	P≤0.001	P≤0.001	P≤0.001
day 21/2°C vs treatment						P=0.955	P≤0.01	P≤0.001	P≤0.001

In all, while fresh, refrigerated (2 days post-slaughter) samples could be separated from all four freeze-treatment groups, our data suggests that extended refrigerated storage in beef reduces the detectability of prior freezing, when using bioimpedance spectroscopy. In addition, freeze storage at a higher temperature of -4°C had a lesser effect on bioimpedance response than freezing at lower temperatures (-14°C/-22°C/-80°C).

IV. CONCLUSION

We have previously shown a high detection capacity for bioimpedance-based authentication of fresh versus frozen-thawed meat for the entire shelf life of pork or chicken meat [2 and unpublished data]. For beef, however, the present study suggests important limitations for bioimpedance-based authentication of refrigerated-only meat. Specifically, fresh label authentication may be compromised in beef with longer shelf life, as well as in samples kept at higher sub-zero temperatures.

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